Nutrición Hospitalaria

Nutr Hosp. 2013;28(3):807-815
ISSN 0212-1611 • CODEN NUHOEQ
S.V.R. 318

Original

A pilot study of folic acid supplementation for improving homocysteine levels, cognitive and depressive status in eating disorders

Viviana Loria-Kohen[1], Carmen Gómez-Candela[1], Samara Palma-Milla[1], Blanca Amador-Sastre[2], Angel Hernanz[3] and Laura M. Bermejo[1]

[1]Nutrition Department. La Paz University Hospital. Health Research Institute IdiPAZ. Madrid. Spain. [2]Psychiatry Department. La Paz University Hospital. Health Research Institute IdiPAZ, Madrid. Spain. [3]Biochemistry Department. La Paz University Hospital. Health Research Institute IdiPAZ, Madrid. Spain.

Abstract

Background & aims: **Several authors have reported low folate intake in patients with eating disorders (ED). This vitamin plays an essential role in synthesis reactions for neurotransmitters and structural elements of neurons, and therefore its deficiency has been associated with the presence of different disorders linked to mental function. The aim of this study was to determine the effect of folic acid supplementation on homocysteine levels and the cognitive and depressive status of a group of patients with eating disorders with low folate intake.**

Subjects/methods: **The study was designed as a randomised, prospective clinical trial, which included 24 participants assigned to two treatment groups for six months: supplemented group (SG) (10 mg/day of folic acid [ACFOL®]) and a placebo group (PG). Both groups maintained their medical, dietary and psychological treatment. At baseline and end of the intervention, anthropometric, dietary and biochemical parameters (plasma homocysteine [Hcy], serum and red blood cell folate) were recorded. Cognitive and depressive status questionnaires were administered (Stroop Test, Trail Making Test and Beck Depression Inventory).**

Results: **Twenty-two patients completed the study (SG: 12, PG: 10, mean age: 24.2 ± 8.8 years, BMI 18.9 ± 3.5 kg/m^2). The SG significantly increased their serum and red blood cell folate levels and lowered Hcy levels (9.4 ± 2.4 μmol/l *vs*. 7.5 ± 1.7 μmol/l, P < 0.01). The SG also significantly improved most of their test scores for cognitive and depressive status. The PG showed no significant changes in any of the evaluated variables.**

Conclusions: **The results show that folic acid supplementation may be used as another tool within the comprehensive and multidisciplinary treatment applied to patients with ED.**

(*Nutr Hosp*. 2013;28:807-815)

DOI:10.3305/nh.2013.28.3.6335

Key words: *Folate. Cognitive function. Eating disorders. Depression. Homocysteine.*

Correspondence: Viviana Loria-Kohen.
Nutrition Department. La Paz University Hospital.
Health Research Institute IdiPAZ.
Paseo de la Castellana, 261.
28046 Madrid, Spain.
E-mail: vloria@hotmail.com

Recibido: 22-XI-2012.
1.ª Revisión: 22-XI-2012.
Aceptado: 29-XI-2012.

ESTUDIO PILOTO SOBRE EL EFECTO DE LA SUPLEMENTACIÓN CON ÁCIDO FÓLICO EN LA MEJOR DE LOS NIVELES DE HOMOCISTEÍNA, FUNCIÓN COGNITIVA Y ESTADO DEPRESIVO EN TRASTORNOS DE LA CONDUCTA ALIMENTARIA

Resumen

Introducción y objetivo: **Diferentes autores han reportado una baja ingesta de ácido fólico en pacientes con Trastornos de la Conducta Alimentaria (TCA). Esta vitamina desempeña un papel esencial en las reacciones de síntesis de neurotransmisores y elementos estructurales de las neuronas y, por lo tanto, su deficiencia se ha asociado con la presencia de diferentes trastornos relacionados con la función mental. El objetivo de este estudio fue determinar el efecto de la suplementación con ácido fólico sobre los niveles de homocisteína y sobre marcadores de función cognitiva y depresión en un grupo de pacientes con TCA con baja ingesta de ácido fólico.**

Sujetos y métodos: **Estudio clínico randomizado y prospectivo en el que se incluyeron 24 pacientes asignados a dos grupos de tratamiento durante un período de 6 meses: grupo suplementado (SG) (10 mg/día de ácido fólico [ACFOL®]) y grupo placebo (PG). Ambos grupos mantuvieron su tratamiento médico, dietético y psicológico. Al inicio del estudio y tras la intervención se evaluaron parámetros antropométricos, dietéticos y bioquímicos (homocisteína plasmática [Hcy], folato sérico y eritrocitario). Como marcadores de función cognitiva y depresión se administraron diferentes cuestionarios (Test de Stroop, Trail Making Test, BDI: Cuestionario de percepción de función cognitiva).**

Resultados: **Completaron el estudio 22 pacientes (SG: 12, PG: 10, edad media: 24,2 ± 8,8 años, IMC 18,9 ± 3,5 kg/m^2). El grupo SG incrementó de forma significativa sus niveles de folato sérico y eritrocitario y redujo el de homocisteína (9,4 ± 2,4 μmol/l *vs*. 7,5 ± 1,7 μmol/l, P < 0,01). Además, el grupo SG también mejoró significativamente las puntuaciones de los test de función cognitiva y depresión. En el grupo PG, en cambio, no se observaron cambios significativos en ninguna de las variables evaluadas.**

Conclusiones: **Los resultados obtenidos demuestran que la suplementación con ácido fólico podría emplearse como una herramienta más dentro del complejo y multidisciplinario tratamiento que requieren estos pacientes.**

(*Nutr Hosp*. 2013;28:807-815)

DOI:10.3305/nh.2013.28.3.6335

Palabras clave: *Folato. Función cognitiva. Trastorno alimentario. Depresión. Homocisteína.*

Abbreviations

ED: Eating Disorders.
RA: Restrictive Anorexia Nervosa.
EDNOS: Eating Disorder Not Otherwise Specified.
PG: Placebo group.
SG: Supplemented group.
Hcy: Homocysteine.
ST: Stroop test.
TMT: Trail Making Test.
BDI: Beck Depression Inventory.

Introduction

Folate deficiency is a common nutritional problem in many populations groups. This deficiency may be due to absorption disorders, genetic factors, drug interactions and inadequate diet.[1]

Several authors have reported low folate intake in patients with eating disorders (ED).[2,3] The severe food restriction of that patients, especially those with Restrictive Anorexia Nervosa (RAN), causes them to have diets deficient in both energy and micronutrients, among them folate.

Folate is the generic term for the various chemical forms of folic acid that can only be synthesised by plants and microorganisms, thus requiring it to be ingested through diet.[4,5] This vitamin plays an essential role in synthesis reactions for neurotransmitters and structural elements of neurons,[6] and therefore its deficiency has been associated with the presence of different disorders linked to mental function such as depression[6-8] and cognitive function impairment.[9] The effect of folic acid supplementation has been studied as a tool for improving these disorders, with conflicting results.[10-13]

Some authors have identified the presence of increased homocysteine levels in eating disorders patients,[14-17] and attempts have been made to find some association between this and the high rates of depression[18,19] and cognitive function impairment recorded in these patients.[20,21] It is still not known what causes this increase in homocysteine levels, whether the levels return to normal after the nutritional state is normalised and what strategies must be employed to carry out this normalisation.[22]

The aim of this study was to determine the effect of folic acid supplementation on homocysteine levels in an ED group of patients. The secondary objectives were to evaluate the outcome on cognitive and depressive status after the intervention.

Subject/methods

Study participants

This study was designed as a prospective, randomised, double-blind, parallel-placebo clinical study, developed by the Nutrition Department of "La Paz University Hospital" in the period between January 2008-June 2010. Twenty-four males and females diagnosed with eating disorders (RAN: Restrictive Anorexia Nervosa and EDNOS: Eating Disorder Not Otherwise Specified) through clinical interviews with the Psychiatry Department and measured for low folate intake, based on the recommended daily intake in terms of age,[23] were included consecutively. Exclusion criteria were: hypersensitivity to folic acid or anemia due to lack of B12, patients who routinely used drugs that interfere with folic acid absorption (analgesics, anticonvulsants, hydantoin, carbamazepine, antacids, antibiotics, cholestyramine, methotrexate, pyrimethamine, triamterene, trimethoprim and sulphonamides) and patients taking vitamin and mineral supplements.

All participants or their relatives, in the case of minors, signed an informed consent for participation. The study was approved by the Ethics Committee of the La Paz University Hospital and conformed to the ethical standards of the Declaration of Helsinki. Registered under ClinicalTrials.gov Identifier no. NCT01493674.

Interventions

Patients were randomly assigned to two treatment groups. The treatment groups consisted of a supplemented group (SG) using two 5-mg tablets of folic acid (ACFOL®), and a placebo group (PG) using two tablets that were identical to those of SG, but composed of crystalline cellulose, lactose and food colouring. Groups were treated for six months. Patients continued the standard medical, dietary and psychological treatment established for these patients within the Nutrition Department.

Methods

The following data were collected at baseline and at the end of the study:

- Anthropometric parameters: height (SECA stadiometer [range: 80 cm to 200 cm]) and weight (TANITA BC-420MA, Biologica Tecnologia Medica S.L, Barcelona, Spain) were measured. BMI was calculated using the equation: weight (kg)/[height (m)]^2.
- Dietary parameters: all food and beverages consumed were recorded using a food frequency questionnaire and a "3-day food and drink record", validated for Spanish population.[24] The food's energy and nutritional content was then calculated using nutrition software (DietSource® 3.0, Novartis, Spain). The values obtained were compared to the recommended values to determine the diets' nutritional adequacy.[23,30]

– Blood variables: hematology determinations were performed using an ABX Pentra 120 autoanalyser (Horiba). Serum and red blood cell folate and vitamin B12 levels were determined by a Modular Analytics E 170 autoanalyser (Roche). Plasma homocysteine was quantified by nephelometry using a Prospec autoanalyser (Siemens). The following reference values were used for the diagnosis of folate deficiency: serum folate < 3 ng/ml, red blood cell folate < 140 ng/ml, Hcy > 8 μmol/l and vitamin B12 < 258 pmol/l.[25]
– Depressive and cognitive status:
  • Depressive symptomatology was assessed using the Beck Depression Inventory (BDI).[26] The BDI is a 21-item self-report scale measuring the depression severity (range 0-63). Depression absence is defined as a total score below 12, moderate depressive symptoms as a score between 12 and 17 and a clinically relevant depression, 18 or above.
  • Selective attention and executive function was measured using the Stroop colour-word interference test.[27] The first part of the test (named P) consists of reading the names of colours printed in black ink, and measures verbal ability and attention. In the second part of the test (named C), participants have to name the colour (blue, green or red) of a series of printed dashes. In the third part (named PC), participants have to name the colour of coloured words printed in incongruent ink colours instead of reading the name. Interference (I) is calculated using the scores from the three test parts and measures the ability to adapt to changing demands and suppress habitual responses in favour of unusual ones [I = PC-(C*P)/(C + P)]. The T score was determined by crossing the variables in the score calculation table.[27] Normal limits for T scores ranged from 35 to 65 points. To assess the various parts of the test, the following scales were used for Spanish population:[28]

Stroop scores	*Test card P*	*Test card C*	*Test card PC*	*Interference*
Adults 16 to 44 years of age (mean values)	119	79	50	2.71

  • Trail Making Test,[29] evaluates visual search speed, attention, visuospatial sequencing, mental flexibility and motor function. The test has two parts: TMT-A (participants have to connect digits from 1 to 25 in ascending order after they have performed a similar training task with only 8 digits), and TMT-B [participants have to connect digits and letters sequentially (1-A, 2-B, … 13-L)]. The test variable was the time in seconds needed to correctly complete the task. In accordance with Frieling et al. (2005), TMT values were scored as paired or unpaired (Cut-off: TMT-A > 40s; TMT-B > 85s).

Prior to completing the cognitive function questionnaires, the patient was checked to make sure they were not fasting and were getting a regular amount of sleep, in order to minimise the effects of these factors on the results.

Other interesting medical parameters were recorded at baseline: age and time of disease diagnosis.

Statistical analysis

Analysis was performed using the SPSS 9.0 programme (SPSS Inc., Chicago, IL, USA). Continuous variables are shown as mean (standard deviation [SD]). Qualitative variables are shown as absolute frequencies and percentages. Due to the sample size, the Shapiro-Wilk test was used for performing normality tests. When the distribution of the results was normal, the Student t test was used to compare the mean values of the studied variables recorded for the two treatment groups. The Mann-Whitney U test was used when the distribution was not normal. Differences within groups at the beginning and end of the study were examined using the Student paired t test when the distribution of the results was normal, and the Wilcoxon test when it was not. We also calculated linear correlation coefficients using the Pearson's test when at least one of the variables was normally distributed, or the Spearman's test when both were not normal. Values of $p < 0.05$ were considered significant for all statistical tests.

Results

Twenty-four patients were recruited (SG: 14, PG: 10). Two patients in the SG withdrew from the Nutrition Department's standard treatment, so the data from their last visit could not be obtained and they were excluded (PG: 12, PG: 10). No side effects were reported during treatment.

At the start of the study there were no significant differences in the values of any of the variables studied (table I). After assessing folate intake by means of the food record, we observed that half of the group did not meet 30% of the recommended daily intake for their age (400 μg/day).

The total study sample presented baseline serum and red blood cell folate and vitamin B12 levels within reference values: 9.7 ± 3.3 ng/ml; 725.6 ± 306.1 ng/ml and 654.0 ± 309.1 pg/ml, respectively. Mean Hcy values were 9.4 ± 2.4 μmol/l. The only male included in the study had 7.8 μmol/l.

In terms of the correlation between Hcy levels and the specific variables linked to metabolism of this

Table I
Socio-sanitary characteristics of the study group. Data expressed as mean (SD) and percentage

	Supplemented group n = 14	*Placebo group n = 10*
Age (years)	22.3 (7.6)	26.7 (10.0)
Evolution time (years)	3.5 (7.4)	7.1 (8.9)
Diagnosis (DSM IV) (%)		
RAN	50.0	42.9
EDNOS	50.0	57.1
Education level (%)		
UE	46.2	66.6
SE	23.0	11.2
PE	30.8	22.2
Consumption of psychoactive drugs (%)		
Antidepressants	57.1	70.0
Anxiolytics	42.9	30.0
Mood stabilisers	7.0	0.0
Dietary intake		
Energy (kcal)	3.96 (1.39)	4.75 (2.32)
Folates (µg/day)	114.5 (51.7)	143.3 (77.2)
% coverage of RDI of folic acid	28.6 (12.9)	35.8 (19.3)
Vitamin B_{12}	2.3 (1.2)	3.1 (2.4)
% coverage of RDI of B_{12}	96.7 (51.1)	130.4 (103.1)

RAN: Restrictive Anorexia Nervosa; EDNOS: Eating Disorder Not Otherwise Specified; UE: University education (completed or in progress); SE: Secondary education (completed or in progress); PE: Primary education; RDI: Recommended Dietary Intake.

amino acid (folate, vitamin B12), only an inverse and weak correlation between homocysteine and vitamin B12 was found ($r = -0.412$ $P < 0.05$).

About cognitive function results at baseline, 8.3% of participants scored below the mean of the reference in P, and 33.3% in C and CP test cards for Stroop test (ST). Some 33.3% scored below the I mean. Only one patient (4%) had a T score outside normal ranges.[27] Regarding TMT, 8.4% scored above the reference cut-off used in part A and 20.8% in part B.[21] About depression symptoms assessment at baseline, 20.8% scored in the moderate range of depression and 58.3% scored in relevant or severe ranges on the BDI.[26]

After six months of intervention, there were no significant changes in caloric or macronutrient intake. In terms of micronutrients, significant differences were only observed in the total folate intake. The SG had a greater increase of this nutrient as a result of supplementation (28.0 ± 65.5 *vs.* 9995.2 ± 58.8 µg/day; $P < 0.001$).

Biochemical parameters evolution after the intervention is shown in table II. The SG significantly increased their serum and red blood cell folate levels and lowered Hcy levels. There were no significant changes in vitamin B12 levels in either treatment group.

The evolution of scores from the different questionnaires is shown in table III. The SG significantly lowered the time spent resolving part B of the TMT and increased the number of words read in the ST test cards P, C and CP. BDI scores were also significantly lower (figs. 1, 2). There were no significant changes in any of the tests assessed for the PG.

The increase in total folate intake in SG correlated significantly and inversely with the change in BDI scores ($r = -0.581$, $P < 0.05$), but not with other questionnaires.

In terms of BMI evolution, the SG significantly increased their BMI (18.9 ± 3.2 *vs.* 20.1 ± 2.6 kg/m^2; $P < 0.05$). PG did not change this parameter (18.8 ± 3.9 *vs.* 18.6 ± 4.0 kg/m^2). Also, the differences between the variations of the groups were significant (SG: 1.4 ± 1.9 *vs.* PG: -0.2 ± 1.2 kg/m^2; $P < 0.05$). There were no significant correlations between BMI changes and in the questionnaires scores changes or in the Hcy changes. The coefficients of determination R^2 indicated that only a low and insignificant percentage of the change in the variation of studied variables may be attributed to the variation in BMI (Stroop P: $R^2 = 0.004$, $P = 0.788$; Stroop C: $R^2 = 0.03$, $P = 0.806$; Stroop PC: $R^2 = 0.035$, $P = 0.406$; TMT-A: $R^2 = 0.079$, $P = 0.206$; TMT-B: $R^2 = 0.044$, $P = 0.347$; BDI: $R^2 = 0.159$ $P = 0.066$; Hcy $R^2 = 0.009$, $P = 0.077$).

When studying the relationship between patient age and time of diagnosis of the disease with the changes at the end of the intervention, there were no significant correlations in any of them.

Discussion

After six months of a 10-mg/day folic acid supplementation in EDs patients, there was an improvement in folate status and a significant reduction in Hcy levels. Additionally, there were significant and favourable changes in most cognitive function and depression test scores.

At baseline of the study, folate intake of all patients was deficient (< 67% RDI), and did not achieve 30% of the recommended daily intakes.[30] These values are similar to previous studies.[2,31]

Folate is involved in various biological functions necessary for achieving a healthy state. Some studies suggest that deficient folate status is associated with high levels of Hcy.[9,32] Hcy levels above 10 µmol/l are linked to a poor cognitive state and depression.[33]

The median for Hcy in our groups [9.6 µmol/l (range: 5.2 to 14.4)] was greater than reference values for the Spanish population [7.79 µmol/l (range: 4.3 to 17.7)].[32] Some 66% of the sample had baseline Hcy levels greater than the reference value considered for indicating folate deficiency (> 8 µmol/l).[1] Moreover, it has been observed that 42% of all the patients had base-

Table II
Evolution of biochemical parameters after intervention. Data expressed as mean (SD)

	Pre-intervention		*Post-intervention*		*Difference*	
	Supplemented group n = 14	*Placebo group n = 10*	*Supplemented group n = 12*	*Placebo group n = 10*	*Supplemented group n = 12*	*Placebo group n = 10*
Homocysteine (µmol/l)	9.4 (2.4)	10.0 (2.05)	7.5 (1.7)[a]**	8.0 (1.8)	-2.0 (1.8)	-1.6 (2.0)
Serum folate (ng/ml)	9.4 (3.6)	10.2 (3.8)	19.6 (1.3) [a]***	10.9 (3.8)	10.6 (3.4)	0.4 (2.0) [b]***
Red blood cell folate (ng/ml)	634.3 (300.0)	844.4 (285.4)	1,521.7 (167.0)[a]***	945.0 (347.0)	919.0 (311.0)	119.1 (101.7)[b]***
Vitamin B12 (pg/ml)	562.6 (209.5)	782.0 (387.0)	580.6 (223.3)	762.8 (468.3)	14.9 (124.6)	34.9 (172.6)
Haematocrit (%)	39.1 (1.8)	39.4 (3.2)	38.8 (2.0)	39.8 (2.0)	-0.8 (1.5)	0.1 (38.0)
Haemoglobin (g/dl)	13.0 (0.6)	13.5 (0.9)	13.0 (0.8)	13.4 (0.9)	-0.2 (0.7)	-0.2 (0.2)

[a]Intra-group differences after 6 months of intervention.
[b]Differences between groups after 6 months of intervention.
Level of significance *P < 0.05, **P < 0.01, ***P < 0.0001.

Table III
Changes in mean scores for cognitive function, depression and perception of cognitive impairments tests after intervention. Data expressed as mean (SD)

	Pre-intervention		*Post-intervention*	
	Supplemented group n = 14	*Placebo group n = 10*	*Supplemented group n = 12*	*Placebo group n = 10*
TMT-A (s)	31.8 (19.0)	26.7 (9.04)	27.5 (11.9)	23.4 (9.3)
TMT-B (s)	78.8 (58.9)	63.8 (39.2)	52.3 (24.0)[a]*	61.3 (42.1)
P-Stroop (nw)	99.8 (19.5)	104.0 (16.0)	105.6 (21.4)[a]*	110.1 (18.2)
C-Stroop (nw)	70.6 (16.8)	65.5 (13)	77.1 (17.3)[a]*	70.0 (16.2)
PC-Stroop (nw)	45.9 (10.8)	44.7 (11.6)	49.2 (11.2)[a]*	47.6 (14.0)
I-Stroop	5.9 (6.9)	4.7 (7.6)	5.1 (6.9)	5.0 (7)
PT-Stroop	55.5 (7.1)	54.0 (6.2)	55.7 (6.1)	54.9 (6.9)
BDI	22.9 (8.1)	17.3 (12.1)	15.2 (9.9)[a]*	13.4 (11.8)

s: seconds; nw: number of words; TMT-A: Trail Making Test part A; TMT-B: Trail Making Test part B; P, C and PC: Stroop test sheets; I: interference; TS: T score; BDI: Beck Depression Inventory.
[a]Intra-group differences after 6 weeks of intervention.
Level of significance *P < 0.05.

line Hcy levels greater than the value indicative of a health risk.[33]

The presence of high Hcy levels has been reported in ED patients.[14-17] However, we should take into account the significant variability in the data collection and analysis methods, such as the control markers used, which must be considered when interpreting and comparing these data.[32,34]

Despite ED patients have a high nutritional risk for some nutrients,[3] both groups in our study had normal blood vitamin B12 and serum and red blood cell folate levels, an observation found in other studies.[14,16]

Vitamin B12 deficiency is rare since the ratio of cobalamin body reserves to its normal daily requirements is approximately 1000:1, which makes it difficult to develop a deficiency in this vitamin based solely on a deficient diet. Its deficiency is caused more by congenital errors in metabolism or by gastrointestinal problems related to surgery, age, etc.[1] Previous studies have not found vitamin B6 deficits in patients with ED, and Hcy levels were not reduced after supplementing with this vitamin.[15] However, some authors suggest that conventional criteria for the folate deficiency diagnosis may be inadequate to identify individuals who may benefit from dietary supplements,[9] since finding appropriate levels would not reflect the actual situation. Therefore, to understand the exclusive effect of folate on the study variables, we decided to perform folic acid supplementation and not in combination with B6 and B12, as was done in many previous studies.[10-12]

Before carrying out an intervention with folic acid, vitamin B12 deficiency had to be ruled out since both nutrients use common metabolic pathways, and supplementation with folic acid may mask B12 deficiency.[9] In our study, folic acid supplementation in the doses and times used did not cause a significant reduc-

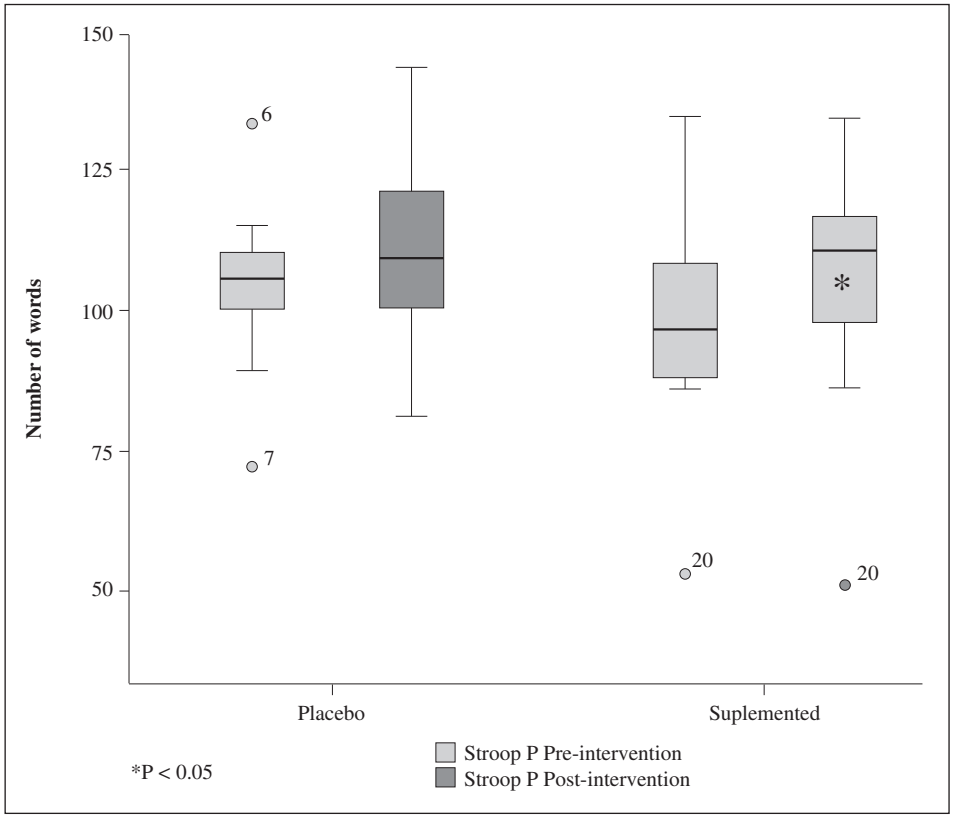

Fig. 1.—The supplemented group (SG) significantly increased the number of words read in the Stroop Test (ST) test cards P after the intervention.

tion in vitamin B12 levels whose baseline values were within normal ranges.

After the intervention, SG showed significant changes in various parameters linked to folate status, increasing both serum and red blood cell folate. Moreover, only SG experienced a drop in Hcy values. Other interventions with folic acid, mainly on elders, also achieved improvements in folate nutritional state as well as significant reductions in Hcy levels.[11-13,35]

EDs are associated with cognitive function impairment.[20,21] Based on studies performed on Alzheimer's patients, which associate increased Hcy with cognitive impairment,[36] it is hypothesised that high Hcy levels may contribute to cognitive impairment in ED patients.

Baseline cognitive status of participants assessed with the Stroop test showed that almost a third of them were below the mean scores for the same age Spanish population.[28] However, baseline Hcy levels were not significantly correlated to test scores. Frieling et al. (2005) assessed the cognitive function of patients with ED using this test and obtained scores and percentages similar to those found at baseline in our study. They also did not find a relationship between Hcy levels and Stroop test scores. These results suggest that Hcy levels may not immediately reflect cognitive function impairment.

After the intervention, only SG improved their cognitive state, significantly increasing the number of words read in the P, C and CP test sheets. The changes in scores for PG were not significant.

After the intervention, SG improved their cognitive state, significantly reducing the time spent in solving TMT part B. As in the previous test, despite the fact that a reduction in test times was expected due to repetition of the test, the changes in the times for the PG were not significant.

It has been suggested that depression symptoms in patients with ED occur due to neuroendocrinological disorders induced by food restriction.[19] According to scores obtained for the BDI, more than three quarters of all participants achieved scores indicative of depression. The prevalence rates for depression reported in ED patients ranged from 35% to 85%, and are greater in patients with RAN.[18,19] Frieling et al. (2008), using the same test, obtained baseline scores that were equal to those of our study and found a significant correlation between baseline Hcy levels and test scores,[19] which was not observed in our study or other recently performed study.[22]

After the intervention, SG improved their depressive state by significantly reducing BDI scores, which was not observed in PG. Although only 7.1% of the SG scored in normal ranges at baseline, the percentage increased to 41.7% after the intervention. The percentage of those who scored in the severe ranges also decreased (78.6% to 33.3%). The evolution of the percentages in the normal range in the PG was from 40% to 60%, and fell 10% for severe cases. We should note that for this analysis both groups received routine psychiatric and psychological treatment, which

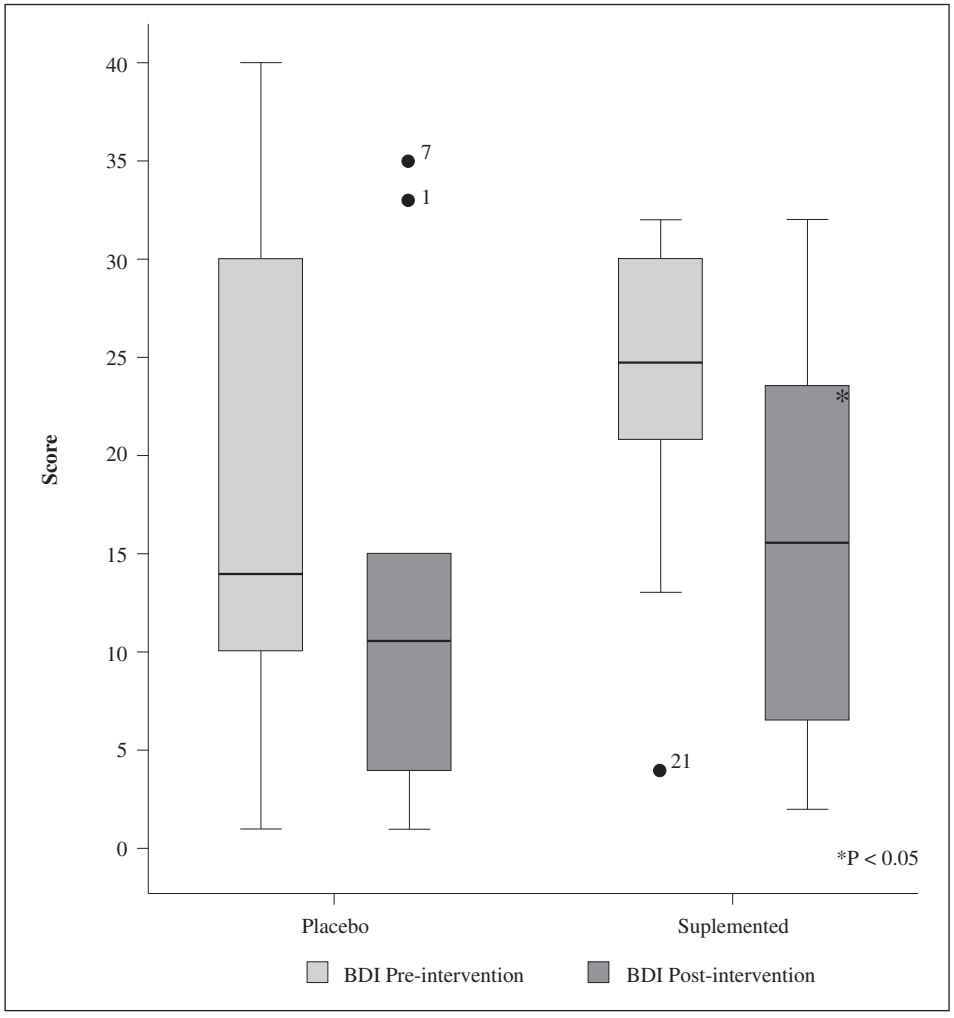

Fig. 2.—The supplemented group (SG) significantly lowered Beck Depression Inventory (BDI) scores after the intervention.

explains the positive evolution of PG. In addition, it was observed that BDI scores changes were significantly correlated with total folate intake changes. Therefore, oral supplementation with folic acid may prove to be beneficial in the treatment of this type of disorder in patients with ED.

We have not found previous studies on ED patients who supplemented with folic acid. However, we can find extensive literature with conflicting results on the influence of folate and folic acid supplementation on cognitive function in individuals with cognitive impairment due to age and dementia. A review by Cochrane concludes that there was no evidence of benefit from folic acid supplementation with or without the addition of B12 compared to placebo in some of the measures of cognition and mood in healthy individuals with cognitive impairment or dementia. However, in a trial that recruited healthy elderly people with high Hcy levels, the administration of folic acid supplements for three years was associated with a significant benefit in overall function, memory capacity and information processing speed. The authors suggest that more studies are necessary in this area.[9] It should be noted that most of the review studies are on elders, which means that some of the neurological lesions may be irreversible despite supplementation.

About the anthropometric parameters evolution after intervention, SG significantly increased their BMI, a condition not observed in PG. Previous studies have shown that nutritional rehabilitation (without vitamin B complex supplementation) may significantly reduce Hcy levels.[14] In contrast, a recent study observed that after nutritional treatment (not specified by the authors) there was a significant increase in BMI and yet Hcy, folate and B12 levels did not change and there were no changes in most of the cognitive function tests used.[22] In our study, the increase in BMI after the intervention was not associated with improved results in both Hcy levels and test scores.

Other determinants of the evolution of cognitive and depressive status, may include age and time of diagnosis of the disease.[16] However, in our study there was no association between them.

This study is the first clinical trial, to our knowledge, that used folic acid supplements to assess changes in cognitive and depressive status in EDs patients. Another

strength was the exclusive folic acid supplementation, not in combination with other B vitamins. This allowed us to isolate the results for this vitamin and to verify the folic acid supplementation use in participants without prior deficiencies does not cause negative effects on vitamin B12 levels. It is also noteworthy that an extensive battery of tests was used to assess the evolution in cognitive and depression status (Stroop, TMT, and BDI), since current evidence suggests that Hcy levels alone cannot report on the clinical repercussion in cognitive and depressive status.

One of the limitations of this study was the small sample size. Nevertheless, other EDs patients publications have similar sample sizes. The small size is consequence of the low prevalence of this disease coupled with the strict exclusion criteria. The small sample size precludes a proper analysis by subgroups, which would have been of great interest. Moreover, the inclusion of a healthy control group would have been of significant value for comparing baseline test results with a reference population.

Positive evolution of the cognitive and depressive status observed in SG may contribute to improving their quality of life and recovery. This fact demonstrates the scientific importance of this study, since folic acid supplementation may be used as tool within a comprehensive and multidisciplinary treatment for ED patients.

Conclusions

Supplementation with 10 mg/day of folic acid for six months in patients with EDs (RAN and EDNOS) and low folate intake produced an improvement in folate status, as well as, significant reduction in Hcy levels and significant and favourable changes in most test scores for cognitive and depressive status. Supplementation was safe and vitamin B12 levels were not affected.

Further studies with larger sample sizes are needed to expand and support these results.

Acknowledgements

We thank the study participants and the staff of the Nutrition Department at La Paz University Hospital who contributed to its successful completion.

This study was made possible thanks to the donation of study tablets and placebos from the laboratory ITALFARMA S.A.

The authors declare no conflict of interest.

References

1. González-Gross M, Sola R, Castillo MJ. Folate revisited. *Med Clin (Barc)* 2002; 119: 627-35.
2. Hadigan CM, Anderson EJ, Miller KK, Hubbard JL, Herzog DB, Klibanski A et al. Assessment of Macronutrient and Micronutrient Intake in Women with Anorexia Nervosa. *Int J Eat Disord* 2000; 28: 284-92.
3. Loria Kohen V, Gómez Candela C, Lourenço Nogueira T, Pérez Torres A, Castillo Rabaneda R, Villarino Marin M, et al. Evaluation of the utility of a Nutrition Education Program with Eating Disorders. *Nutr Hosp* 2009; 24: 558-67.
4. Moreiras-Varela O, Nunez C, Carbajal A, Morande G. Nutritional Status and Food Habits Assessed by Dietary Intake and Anthropometrical Parameters in Anorexia Nervosa. *Int J Vitam Nutr Res* 1990; 60: 267-74.
5. Fernstrom MH, Weltzin TE, Neuberger S, Srinivasagam N, Kaye WH. Twenty-Four-Hour Food Intake in Patients with Anorexia Nervosa and in Healthy Control Subjects. *Biol Psychiatry* 1994; 36: 696-702.
6. Karakuła H, Opolska A, Kowal A, Doma ski M, Płotka A, Perzy ski J. Does diet affect our mood? The significance of folic acid and homocysteine. *Pol Merkur Lekarski* 2009; 26: 136-41.
7. Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, Vitamin B12, Homocysteine, and the MTHFR 677C->T Polymorphism in Anxiety and Depression: the Hordaland Homocysteine Study. *Arch Gen Psychiatry* 2003; 60: 618-26.
8. Papakostas GI, Petersen T, Mischoulon D, Green CH, Nierenberg AA, Bottiglieri T et al. Serum Folate, Vitamin B12, and Homocysteine in Major Depressive Disorder, Part 2: Predictors of Relapse During the Continuation Phase of Pharmacotherapy. *J Clin Psychiatry* 2004; 65: 1096-8.
9. Malouf M, Grimley EJ, Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia. Cochrane Database Syst Rev 2008: published online Jul 16 DOI: 10.1002/14651858.CD004514.
10. Bryan J, Calvaresi E, Hughes D. Short-Term Folate, Vitamin B-12 or Vitamin B-6 Supplementation Slightly Affects Memory Performance but not Mood in Women of Various Ages. *J Nutr* 2002; 132: 1345-56.
11. Clarke R, Harrison G, Richards S; Vital Trial Collaborative Group. Effect of Vitamins and Aspirin on Markers of Platelet Activation, Oxidative Stress and Homocysteine in People at High Risk Of Dementia. Clarke R, Harrison G, Richards S; Vital Trial Collaborative Group. *J Intern Med* 2003; 254: 67-75.
12. McMahon JA, Green TJ, Skeaff M, Knight RG, Mann JI, Williams SM. A Controlled Trial of Homocysteine. Lowering and Cognitive Performance. *N Engl J Med* 2006; 354: 2764-72.
13. Durga J, van Boxtel MP, Schouten EG, Kok FJ, Jolles J, Katan MB et al. Effect of 3-Year Folic Acid Supplementation on Cognitive Function in Older Adults in the FACIT Trial: a Randomised, Double Blind, Controlled Trial. *Lancet* 2007; 369: 208-16.
14. Moyano D, Vilaseca MA, Artuch R, Valls C, Lambruschini N. Plasma Total-Homocysteine in Anorexia Nervosa. *Eur J Clin Nutr* 1998; 52: 172-5.
15. Frieling H, Römer K, Röschke B, Bönsch D, Wilhelm J, Fiszer R et al. Homocysteine Plasma Levels are Elevated in Females With Anorexia Nervosa. *J Neural Transm* 2005; 112: 979-85.
16. Levine J, Gur E, Loewenthal R, Vishne T, Dwolatzky T, van Beynum IM et al. Plasma homocysteine levels in female patients with eating disorders. *Int J Eat Disord* 2007; 40: 277-84.
17. Innis SM, Birmingham CL, Harbottle EJ. Are Plasma Homocysteine and Methionine Elevated When Binging and Purging Behavior Complicates Anorexia Nervosa? Evidence Against the Transdiagnostic Theory of Eating Disorders. *Eat Weight Disord* 2009; 14: 184-9.
18. O'Brien KM, Vincent NK. Psychiatric Comorbidity in Anorexia and Bulimia Nervosa: Nature, Prevalence, and Causal Relationships. *Clin Psychol Rev* 2003; 23: 57-74.
19. Frieling H, Römer KD, Beyer S, Hillemacher T, Wilhelm J, Jacoby GE et al. Depressive symptoms may explain elevated plasma levels of homocysteine in females with eating disorders. *J Psychiatr Res* 2008; 42: 83-6.

20. Lena SM, Fiocco AJ, Leyenaar JK. The Role of Cognitive Deficits in the Development of Eating Disorders. *Neuropsychol Rev* 2004; 14: 99-113.
21. Frieling H, Röschke B, Kornhuber J, Wilhelm J, Römer KD, Gruss B et al. Cognitive impairment and its association with homocysteine plasma levels in females with eating disorders - findings from the HEaD-study. *J Neural Transm* 2005; 112: 1591-8.
22. Wilhelm J, Müller E, de Zwaan M, Fischer J, Hillemacher T, Kornhuber J et al. Elevation of Homocysteine Levels is Only Partially Reversed After Therapy in Females With Eating Disorders. *J Neural Transm* 2010; 117: 521-7.
23. Institute of Medicine. Dietary Reference Intakes: Applications in Dietary Assessment, 2000 and Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients). National Academy Press Washington, D.C., 2002. (Accessed December 5, 2011, at http// www.nap.edu.).
24. Ortega RM, Requejo AM, López-Sobaler AM. Questionniares for Dietetic Studies and the Assessment of Nutritional Status. In: Requejo AM, Ortega RM, Eds. Nutriguía. Manual of Clinical Nutrition in Primary Care. Madrid: Editorial Complutense, 2003: 456-9.
25. Gibson RS. Assessment of the status of folate and vitamin B12. In: Gibson RS, Ed. Principles of nutritional assessment. New York (NY): Oxford University Press; 1990: 461-86.
26. Beck At, Ward Ch, Mendelson M, Mock J, Erbaugh J. An Inventory for Measuring Depression. *Arch Gen Psychiatry* 1961; 4: 561-71.
27. Golden CJ. Stroop Color and Word Test. A Manual for Clinical and Experimental Uses. Wood Dale, Illinois: Stoelting co, 1978.
28. Golden, C.J. Stroop: Test de colores y palabras; traducción y adaptación versión española normalizada. Madrid: TEA Ediciones, 1994.
29. Reitan RM. Trail Making Test – Manual for Administration and Scoring, 2 edn. Tucson, Arizona EE.UU: Reitan Neuropsychology Laboratory, 1992.
30. Institute of Medicine. Dietary Reference Intakes: For Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. National Academy Press Washington, D.C., 1998. (Accessed December 5, 2011, at http// www.nap.edu.).
31. Loria Kohen V, Gomez Candela C, Lourenço Nogueira T, Castillo Rabaneda R, García Huerta M, Zurita L. "Nutritional Education Program Utility in Eating Disorders". *Nutr Clin Diet Hosp* 2007; 27: 7-17.
32. Pijoán Zubizarreta J, Irigoien Garbizu I, Aguirre Errasti C. Population reference ranges and determinants of plasma homocysteine levels. *Med Clin (Barc)* 2001; 117: 487-91.
33. Ganji V, Kafai MR. Third National Health and Nutrition Examination Survey. Demographic, Health, Lifestyle, and Blood Vitamin Determinants of Serum Total Homocysteine Concentrations in the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Clin Nutr* 2003; 77: 826-33.
34. Soberón M, Charaja A, Agüero Y, Oriondo R, Sandoval M, Núñez M. Distribution of plasma homocysteine, folate and B-12 vitamin in Lima, Peru's young adults. Anales de la Facultad de Medicina [online] 2004, 65 (Accessed December 9, 2011, at http://redalyc.uaemex.mx/redalyc/src/inicio/ArtPdfRed.jsp?iCve=37965202> ISSN 1025-5583).
35. Bermejo LM, Aparicio A, Rodríguez-Rodríguez E, López-Sobaler M, Andrés P, Ortega RM. Dietary Strategies for Improving Folate Status in Institutionalized Elderly Persons. *Br J Nutr* 2009; 101: 1611-5.
36. Sachdev P, Parslow R, Salonikas C, Lux O, Wen W, Kumar R et al. Homocysteine and the brain in midadult life: evidence for an increased risk of leukoaraiosis in men. *Arch Neurol* 2004; 61: 1369-76.